Intrasession Reproducibility of RNFL Thickness Measurements Using SD-OCT in Eyes With Keratoconus

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■ **BACKGROUND AND OBJECTIVE:** To evaluate the intrasession reproducibility of the peripapillary retinal nerve fiber layer (RNFL) thickness measurements obtained by spectral-domain optical coherence tomography (SD-OCT) in eyes with keratoconus and normal eyes.

■ **PATIENTS AND METHODS:** Peripapillary RNFL thickness measurements with SD-OCT were repeated three times during the same visit using the eye tracker and retest function in one eye of each participant. Reproducibility was evaluated using within-subject standard deviation (Sw), coefficient of variation (CV), and intraclass correlation coefficient (ICC).

■ **RESULTS:** For the overall global RNFL thickness, the values of the three parameters were Sw (± 1.96 standard error) 1.43 ± 0.24, CV 1.28%, ICC (95% confidence interval) 0.969 (range: 0.947–0.983) in control eyes and Sw (± 1.96 standard error) from 1.41 ± 0.26 to 1.57 ± 0.34, CV from 1.18% to 1.37%, and ICC (95% confidence interval) from 0.951 (range: 0.909–0.976) to 0.977 (range: 0.938–0.993) in eyes with keratoconus.

■ **CONCLUSION:** Measurement of peripapillary RNFL thickness by SD-OCT shows a good intrasession reproducibility in eyes with keratoconus.

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INTRODUCTION

Glaucoma is a chronic degenerative optic neuropathy in which retinal ganglion cells die, leading to gradual vision loss and ultimately blindness.[1] Elevated intraocular pressure (IOP) is a major risk factor for the onset of glaucoma.[2,3] Other risk factors include age, family history, and race.[4] Due to the limited under-

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standing of the molecular mechanisms of the disease, so far IOP has been the only clinically modifiable causative factor, and all current medical and surgical treatments of glaucoma are aimed at reducing IOP.[1]

Keratoconus is an ectatic corneal disorder characterized by progressive corneal thinning that results in corneal protrusion, irregular astigmatism, and decreased vision.[5] Glaucoma or ocular hypertension may coexist in patients with structurally abnormal corneas such as keratoconus.[6] Monitoring of glaucoma in such patients may be difficult because the assessment of IOP is affected by corneal thickness, which is irregularly reduced in keratoconus, and ocular surface disease.[7] Visual field examination can also be affected by refractive changes.

Measurement of the retinal nerve fiber layer (RNFL) thickness is important for the early diagnosis and determination of glaucoma progression.[8] Thinning of the RNFL correlates highly with, or even precedes, visual field loss.[9-12] Therefore, establishing reliable methods of RNFL measurement could be one key step in early diagnosis and treatment of glaucoma.

Optical coherence tomography (OCT) is a noninvasive, cross-sectional imaging technique that allows measurement of RNFL thickness.[13] OCT has been shown to be a highly reproducible imaging modality[14,15] that correlates with ex vivo histologic measurements of the retina.[16,17] To date, patients with astigmatism of more than 5 diopters have been excluded from OCT studies because of the possible effect of corneal alteration on RNFL thickness measurement.[18,19]

Time-domain OCT is a third-generation modality that has a resolution of 8 to 10 µm and is capable of differentiating between healthy and glaucomatous eyes.[20,21] Recent spectral-domain OCT (SD-OCT) technology provides better scan resolution and allows for a greater number of scans acquired at a faster rate than time-domain OCT technology.[22,23] Also, an online eye-tracking device (eye tracker) that compensates for involuntary eye movements during the scanning process and a retest function that ensures follow-up measurements are taken from the same area as the baseline examination[19,24] have been introduced in Spectralis SD-OCT (Heidelberg Engineering GmbH, Heidelberg, Germany). By such devices, improved reproducibility has been reported in healthy subjects.[19,24,25]

The purpose of this study was to determine the reproducibility of peripapillary RNFL thickness measurements obtained with Spectralis OCT using both the eye tracker and retest function in normal eyes and eyes with keratoconus on the same day (intrasession) by a single operator.

PATIENTS AND METHODS

Participants

This was an observational, prospective study. It was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from each participant.

All study participants with keratoconus were recruited consecutively from the outpatient service of the Department of Ophthalmology of the University of Catania between April 2010 and July 2011. The healthy control participants were recruited in the same period from hospital staff with no evidence of disease of any nature, including neurologic disorders.

All participants underwent a full ophthalmic examination including measurement of manifest refraction (sphere and cylinder), determination of Snellen best-corrected visual acuity (BCVA) and intraocular pressure (IOP) by Goldmann applanation tonometry, biomicroscopy of the anterior and posterior segments, optic disc and fundus evaluation, achromatic automated perimetry using the Swedish Interactive Threshold Algorithm Standard, 24-2 program with Humphrey visual field analyzer (Carl Zeiss Meditec, Inc., Dublin, CA), corneal topographic analysis by the Orbscan IIz (Bausch & Lomb, Rochester, NY), and peripapillary RNFL thickness measurements using the Spectralis OCT (Spectralis software version 4.0).

Participants wearing contact lenses for the correction of the refractive error were instructed to discontinue their use before the examination, for at least 2 weeks for soft contact lenses and at least 4 weeks for rigid gas-permeable contact lenses.

The diagnosis of keratoconus was based on corneal topography and slit-lamp observation. In all cases, clinical findings characteristic of keratoconus were evident: corneal topography revealing an asymmetric bowtie pattern, with or without skewed axes, and at least one keratoconus sign on slit-lamp examination, such as stromal thinning, conical protrusion of the cornea at the apex, Fleischer ring, Vogt striae, or anterior stromal scar.[5] The Amsler–Krumeich classification system[26,27] was used to grade keratoconus. For inclusion

in the study, eyes with keratoconus had to have a stage from 1 to 3.

The inclusion criteria for both healthy participants and those with keratoconus were: Snellen BCVA of 0.5 or better, a refractive error lower than ± 5.00 diopters spherical, and clear ocular media (nuclear opalescence, nuclear color, and cortical changes up to grade 3 on the Lens Opacities Classification System III).[28] Participants with other ocular pathologies affecting the cornea, previous uveitis, ocular surgery or trauma, retinal or macular pathology, tilted discs, peripapillary atrophy, and any neurological disease were excluded from the study. If both eyes of one participant fitted inclusion and exclusion criteria, one eye was selected randomly by using a random number generator statistical table.

Image and Data Acquisition

All peripapillary RNFL thickness measurements were performed by the same experienced operator (MR) with the Spectralis OCT using a circular scan pattern (Spectralis software version 4.0). The scan circle was 12° in diameter, which equates to a retinal diameter of 3.5 mm when assuming a standard corneal curvature of 7.7. All measurements were performed in mydriasis.

Within one session, three measurements were taken with the eye tracker and retest function engaged. With the Automatic Real-Time function activated, multiple frames (16) of the same scanning location are performed during the scanning process and images are averaged for speckle noise reduction. As described by Wu et al.,[24] in each participant, a circular peripapillary scan was acquired first and then defined as a reference scan (first scan). Then the camera was restarted, the follow-up button in the acquisition window was pressed, an earlier reference image was selected, and another circular peripapillary scan (second scan) at the same location as the reference image was obtained. This follow-up function was then repeated for the third image (third scan). By this method, three circular scans of the peripapillary RNFL were obtained at exactly the same location.

Between each measurement, the participant was instructed to lean back before being repositioned on the headrest and the correction for spherical error was readjusted. No manual correction was applied to the OCT output. An internal fixation target was used because it has been shown to give the highest reproducibility.[29]

For this study, scans with a quality of less than 15 (as suggested by the manufacturer) were excluded and were repeated until good quality was achieved. If the quality of the scans was less after three attempts, the participant was excluded from the analysis. Likewise, scans with blinks during the scanning process were excluded and repeated.

Statistical Analysis

Demographic and ocular characteristics of the healthy participants and those with keratoconus were compared using analysis of variance (ANOVA) and, in case of significance, the Tukey–Kramer test.

The reproducibility of RNFL thickness measurements was assessed by calculating, for each of the overall global RNFL parameters, four quadrants (superior, temporal, inferior, and nasal), four sectors (temporal superior, temporal inferior, nasal superior, and nasal inferior), and three parameters (the within-subject standard deviation [Sw], the coefficient of variation [CV], and the intraclass correlation coefficient [ICC]). The Sw is the common standard deviation of the repeated measurements and was calculated as the square root of the average of the variances of the measurements of each participant.[30] The CV is a ratio of the standard deviation over the mean and was calculated as the square root of the residual mean squared values of three measures, divided by the mean. ICC was determined by an SPSS Reliability Analysis (SPSS, Inc., Chicago, IL) that uses a one-way random model.[31]

Spearman rank correlation coefficient analyses were used to assess the correlation of the standard deviation of the three repeated measures for each participant with the specific RNFL thickness measurement. Statistical analyses were done using SPSS version 15.0 (SPSS, Inc., Chicago, IL). A P value of less than .05 was considered statistically significant.

RESULTS

Reliable measurements were obtained from 111 participants: 36 control eyes, 26 eyes with grade 1 keratoconus, 38 eyes with grade 2 keratoconus, and 11 eyes with grade 3 keratoconus.

Table 1 shows the demographics and characteristics of the study sample. Among groups, no difference was seen in mean age; astigmatism was greater in eyes with keratoconus ($P < .001$, ANOVA; $P < .01$, Tukey–Kramer among all groups).

Table 2 shows the peripapillary mean RNFL thick-

TABLE 1
Demographics and Characteristics of the Eyes With Keratoconus (Stage I, II, and III) and Control Eyes

Characteristic	Control Eyes (n = 36)	Eyes With Keratoconus		
		Stage I (n = 26)	Stage II (n = 38)	Stage III (n = 11)
Age (y), mean ± SD	29 ± 4	27 ± 8	28 ± 6	30 ± 7
Gender				
Male	16	16	27	7
Female	20	6	11	4
Astigmatism (diopters), mean ± SD	0.3 ± 0.5	2.6 ± 1.2	3.7 ± 1.7	7.3 ± 1.9

SD = standard deviation.

TABLE 2
Peripapillary RNFL Thickness (Microns, Mean ± SD) Detected by SD-OCT in Eyes With Keratoconus (Stage I, II, and III) and Control Eyes

Peripapillary Sectors	Control Eyes (n = 36)	Eyes With Keratoconus		
		Stage I (n = 26)	Stage II (n = 38)	Stage III (n = 11)
Overall global	98 ± 8	96 ± 7	99 ± 8	96 ± 9
Nasal superior	98 ± 17	100 ± 20	101 ± 21	103 ± 22
Nasal	78 ± 12	72 ± 10	79 ± 13	79 ± 12
Nasal inferior	110 ± 16	106 ± 21	109 ± 16	99 ± 21
Temporal inferior	147 ± 17	147 ± 16	153 ± 17	144 ± 18
Temporal	69 ± 8	69 ± 11	70 ± 10	71 ± 12
Temporal superior	134 ± 14	127 ± 18	134 ± 17	126 ± 19

RNFL = retinal nerve fiber layer; SD = standard deviation; SD-OCT = spectral-domain optical coherence tomography.

ness values of the control and keratoconus groups for overall global and sector RNFL thickness. No significant difference in mean RNFL thickness values at all locations was identified in eyes with keratoconus compared with control eyes. Table 3 shows the reproducibility of Spectralis OCT peripapillary RNFL thickness measurements for all study participants.

Within-subjects standard deviation (Sw ± 1.96 standard error) of the overall global measure ranged from 1.41 ± 0.26 (stage II) to 1.57 ± 0.34 (stage I); for measurements in sectors, it ranged from 1.31 ± 0.30 (temporal, stage I) to 4.68 ± 1.95 (nasal superior, stage III) (Table 3). CV for overall global value ranged from 1.18% (stage II) to 1.37% (stage III); for measurements in sectors, it ranged from 1.56 (temporal, stage I) to 4.00 (nasal inferior, stage III) (Table 3). ICC for overall global value ranged from 0.951 (stage I) to 0.977 (stage III); for measurements in sectors, it ranged from 1.31 ± 0.30 (temporal, stage I) to 4.68 ± 1.95 (nasal superior, stage III) (Table 3).

No significant correlation was found between mean RNFL thickness and within-subject variability in any location for all groups (no *P* values < .05, Spearman rank correlation) (Table 4).

DISCUSSION

Results of our study show that measurement of RNFL thickness by Spectralis OCT in eyes with keratoconus is highly reproducible (Sw ranging from 1.41 ± 0.26 to 1.57 ± 0.34) and repeatable (ICCs ranging from 0.957 to 0.977), with values of the statistical parameters investigated similar to those of control eyes.

The values detected in control eyes (Sw of 1.46 microns, mean CV of 1.4, ICC of 0.994) are consistent with those reported in previous studies with Spectralis

TABLE 3
Reproducibility of Retinal Nerve Fiber Layer Thickness Measurements for Eyes With Keratoconus and Control Eyes for Each Peripapillary Sector

		Eyes With Keratoconus		
Peripapillary Sector	**Control Eyes (n = 36)**	**Stage I (n = 26)**	**Stage II (n = 38)**	**Stage III (n = 11)**
Within-subjects standard deviation				
Overall global	1.43 ± 0.24	1.57 ± 0.34	1.41 ± 0.26	1.45 ± 0.53
Nasal superior	2.63 ± 0.49	3.72 ± 0.87	3.77 ± 0.84	4.68 ± 1.95
Nasal	1.92 ± 0.38	2.40 ± 0.62	1.75 ± 0.35	3.48 ± 1.49
Nasal inferior	4.19 ± 0.74	4.27 ± 0.96	4.34 ± 0.89	4.56 ± 1.57
Temporal inferior	3.31 ± 0.58	3.37 ± 0.78	4.41 ± 1.02	4.60 ± 1.76
Temporal	1.52 ± 0.26	1.31 ± 0.30	2 ± 0.44	1.69 ± 0.69
Temporal superior	2.58 ± 0.46	3.27 ± 0.89	4.28 ± 0.99	3.60 ± 1.46
Coefficient of variation				
Overall global	1.28	1.37	1.18	1.25
Nasal superior	2.21	3.21	2.81	3.24
Nasal	1.99	2.53	1.81	3.36
Nasal inferior	3.28	3.38	3.04	4.00
Temporal inferior	1.96	1.91	1.95	2.62
Temporal	1.92	1.56	2.04	1.77
Temporal superior	1.62	1.96	2.23	2.05
Intraclass correlation coefficient				
Overall global	0.969 (0.947–0.983)	0.951 (0.909–0.976)	0.972 (0.953–0.984)	0.977 (0.938–0.993)
Nasal superior	0.977 (0.961–0.987)	0.968 (0.940–0.984)	0.969 (0.948–0.983)	0.956 (0.886–0.987)
Nasal	0.976 (0.958–0.987)	0.942 (0.893–0.972)	0.981 (0.968–0.989)	0.924 (0.812–0.977)
Nasal inferior	0.934 (0.889–0.963)	0.959 (0.924–0.980)	0.932 (0.887–0.961)	0.954 (0.883–0.986)
Temporal inferior	0.961 (0.934–0.978)	0.954 (0.915–0.978)	0.931 (0.886–0.961)	0.941 (0.852–0.982)
Temporal	0.964 (0.939–0.980)	0.987 (0.976–0.994)	0.958 (0.929–0.976)	0.981 (0.950–0.994)
Temporal superior	0.966 (0.942–0.994)	0.968 (0.940–0.984)	0.941 (0.902–0.967)	0.996 (0.911–0.990)

OCT with the use of eye tracker and retest function. For global overall value, Wu et al.[24] found an Sw of 1.34 ± 0.20 microns, mean CV of 1.40, and ICC of 0.990, Langenegger et al.[19] reported a CV of 1% and an ICC of 0.99, and Garcia Martin et al.[25] found a CV of 1.31% with an ICC of 0.987.

Several factors are known to affect the reproducibility of RNFL thickness measurements: pupil dilation,[15] variations of signal strength,[32,33] sampling density,[34] media opacity, and the quadrants measured.[35] In this study, we selected all eyes with good visual acuity, patients who were able to fixate, and eyes in which the quality of the scan was at least 15. Our results show that measurement of RNFL thickness is reproducible in all stages of keratoconus, suggesting that the deformation of the cornea, when not affecting the quality of the image or the fixation, does not alter the reproducibility of measurement. However, the effect on RNFL detected values must be established. Recent studies have found that astigmatism has an effect on RNFL measurement. In particular, with-the-rule astigmatism decreased average, superior, and 12 to 6 sector thickness, whereas against-the-rule astigmatism reduced thickness in the nasal and temporal quadrants.[36]

In keratoconus, the irregular astigmatism could alter the RNFL thickness in some sectors. Similar to other studies, we found higher variability in sector measurement. In general, the narrower the peripapillary area measured, the higher the variability; as the area measured gets larger, more individual measure-

TABLE 4
Values of Spearman Rank Correlation Coefficients Between the Mean and the Standard Deviations of the Three Repeated Measurements RNFL Thickness Values

			Eyes With Keratoconus					
	Control Eyes (n = 36)		Stage I (n = 26)		Stage II (n = 38)		Stage III (n = 11)	
Peripapillary Sector	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Overall global	-0.113	.511	0.163	.427	0.036	.830	-0.350	.292
Nasal superior	0.230	.178	-0.159	.438	-0.021	.902	0.388	.238
Nasal	0.129	.454	0.150	.465	0.098	.557	-0.009	.979
Nasal inferior	0.011	.949	0.133	.516	0.224	.177	-0.055	.873
Temporal inferior	-0.190	.268	-0.121	.555	0.272	.099	-0.324	.331
Temporal	0.016	.924	0.206	.313	0.243	.142	0.392	.233
Temporal superior	0.292	.084	-0.221	.279	0.003	.986	0.497	.120

RNFL = retinal nerve fiber layer.

ments are added into the mean for that area and this type of signal averaging results in more reliable measurements.[35]

In our study, for measurement in sectors, Sw ranged from 1.31 ± 0.30 (temporal, stage I) to 4.68 ± 1.95 (nasal superior, stage III). These values are consistent with the data of Wu et al., which ranged from 1.83 ± 0.27 (temporal quadrant and nasal inferior sector) to 2.39 ± 0.35 (in temporal superior and temporal inferior sectors).[32]

CV values in our study (from 1.56 [temporal, stage I] to 4.00 [nasal inferior, stage III]) are consistent with those reported by Garcia Martin et al.[25] (from 2.67 in inferotemporal area,to 4.04 in nasal area), Langenegger et al.[19] (from 1.4 in temporal superior and temporal inferior sectors to 3 in papillomacular bundle), and Wu et al.[32] (from 1.66 in inferior quadrant to 2.59 in temporal quadrant).

We report ICC values ranging from 0.924 (nasal sector of stage II) to 0.996 (temporal superior sector, stage III), which are slightly greater than those reported by Garcia-Martin et al.[25] (from 0.888 in inferonasal sector to 0.984 in superotemporal sector), Langenegger et al.[19] (from 0.93 in papillomacular bundle to 0.99 in temporal, nasal, and nasal superior sectors), and Wu et al.[32] (from 0.977 in temporal quadrant to 0.990 in nasal inferior sector). However, all values indicate high reproducibility.

This study has several limitations, including the small number of participants, all measurements were performed in the same visit, and patients were asked to stop contact lens use for 2 to 4 weeks before their visit. It is possible that repeated measurements performed at different visits could be affected by a more recent use of contact lenses.

In glaucomatous eyes with progression of visual field alterations, a reduction of 4.3 microns in average RNFL thickness has been found by Stratus OCT. Wu et al. reported that because the Sw of Spectralis OCT ranges from 1.14 to 2.39 mm, this instrument has the sensitivity for detecting glaucomatous changes in longitudinal studies.[32] In eyes with keratoconus in our study, Sw for overall global thickness ranged from 1.41 ± 0.26 (stage II) to 1.57 ± 0.34 (stage I). It is unknown whether changes with corneal morphology could affect RNFL thickness measurement; longitudinal studies in eyes with progressive keratoconus are required to evaluate this aspect.

Measurement of RNFL thickness in eyes with stage I, II, and III keratoconus is highly reproducible. Further studies are required to evaluate the effect of the irregular astigmatism on RNFL value detected and the effect or keratoconus progression on measurements.

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